# Evaluation of Genotype x Environment Interaction on Morphological Characteristics of Eight Selected *Labisia pumila* var. *alata* Clones (Kacip Fatimah) by Francis and Kannenbergs Method

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***Abstract*—***Since Labisia pumila species are not yet produce locally, it is important for this species to undergo propagation and testing at different environment in different location so that only variety or clones that has good mean plant growth over a wide range of environments can be identified as well as introduced to Malaysia farmer. In other means, the selected clones of L. pumila produced are genetically superior and genetically stable. The aim of this study is to evaluate interaction of genotype x environment between eight selected Labisia pumila var. alata clones and to identify stability of these eight clones respected to plant growth characteristics; plant height, leaf number, leaf length, leaf width, and collar region. Eight clones (KF01, KF02, KF03, KF04, KF05, KF06, KF07 and KF08) had tested at four different locations of FRIM Research Station; SPF Mata Ayer, Perlis; SPF Maran, Pahang; SPF Setiu, Terengganu; and FRIM Kepong, Selangor. The experimental unit consisted of 3 blocks, 0.7 m between clone and 0.4 m within rows in randomized complete block design (RCBD) at area of 0.04 ha under a drip irrigation system. Clones main effect from ANOVA analysis was found in give highly contribution for the phenotypic expression of this species at each locations. Across locations, highly significant was found for clone and location / (GXE) effects. The interaction existed raised the need to use stability analysis by Francis and Kannenberg's method to predict the stability. Results scattergram from Francis and Kannenberg's method showed that clones KF07 and KF08 were shows its superiority in growth performances as both present in Group I. Thus, both were selected as potential elite planting materials as they considered stable and perform well in various range of environments at four locations trial.*

***Keywords*— *Environments*, *Labisia pumila, plant growth, scattergram, stability.***

## I. INTRODUCTION

*Labisia pumila* or locally known as Kacip Fatimah is a native plant of Malaysia that commonly found in shady areas of the Forest Reserve at elevations of 80 to 100 m or more. Labisia pumila that fall under family Myrsinaceae also are recognized and identified locally as Selusuh Fatimah, Rumput Siti Fatimah, Akar Fatimah, Pokok Pinggang, and Belangkas Hutan. This herb has been selected as one of the five main herbs under initial phase of the Entry Point Project (EPPs) that implemented through National Key Economic Areas (NKEAs). This project is focused towards improving the product quality and marketing efforts of dietary and herbal supplements to tap the global demand for high-value herbal supplements and remedies. This showed that *L. pumila* getting high demanded from local as it can be treat variety of ailments [4]. Traditionally, the whole *L. pumila* plants are boiled in water and taken as orally for easing the childbirth. Recently, this herb is sold commercially in the form of herbal tea, powder, capsules and tablets in many countries [2]. The demand to this herb is increased through all the year as many researchers found it have anticancer, antioxidant, anti-osteoporosis and anti-inflammatory properties [8][6].

However, raw materials for product development and local uses is not sufficient due to less contribution of the local farmer to cultivate this herb. According to [1] in Globinmed database, less than 10% of the raw materials are cultivated locally. The rest are either imported from overseas or collected from the wild. This situation will lead to adulteration with low quality materials. Since this species are not yet produce locally, it is important for this species to undergo the testing at different environment at different location so that only variety or clones that has good mean performance over a wide array of environments can be identified and introduced to the Malaysia farmer. In other means, the selected clones of *L. pumila* produced are genetically superior and genetically stable.

Since cultivation *L. pumila* needs the most critical environmental conditions such as heavy shade which a least 60%, high soil humidity and no water logging, it is probably exposed to the influence of Genotype x Environment (GxE) Interaction. Sprague [9] indicated that GxE interaction constitutes an important limiting factor in the estimation of variance components and in the efficiency of selection programmes. The presence of a significant GxE interaction for morphological characteristics of *L. pumila* either in plant height, leaf number, leaf length, leaf width and collar region will bring a problem for selecting superior genotypes for elite clones. According to [3], the presence of GxE interaction also disturb the relative performance of genotypes in different environments. Some genotypes will perform well in few environments and very poor in other environments, showing better mean performance across environments. In other cases, some genotypes may have average performance which is more stable over wide environments. Thus, it is important to conduct the evaluation of GxE interaction in order to track the pattern and magnitude of GxE interaction for better understanding of the response of different genotypes to varying environments. For non-significant effect, the stability analysis also will be further analyze for identification of stable and widely adapted and unstable but specifically adapted genotypes. This analysis is so important for clone and variety recommendation. A stable *L. pumila* clones will be selected if it has capable of utilizing the resources available in high yielding environments and has a mean performance that is above average in all environments.

As an easy way of interpretation of responses of genotypes to environmental variation, non-parametric approach was used as it have some advantages over the parametric approach. This approach separates genotypes based on their similarity of response to a range of an environment. This essential information is required for many applications such as selection in plant breeding programs and cultivar testing trials.

The objectives of this study were to i) determine the magnitude of genotype x environment interaction for plant height, leaf number, leaf length, leaf width and collar region; ii) to identify stability of genotypes respective to plant growth characteristics evaluated.

## II. MATERIAL AND METHOD

Eight *L. pumila* var. *alata* clones were used for the study; clone KF01, KF02, KF03, KF04, KF05, KF06, KF07 and KF08. The materials were obtained from Forest Research Institute Malaysia (FRIM)'s germplasm and being screened randomly based on Total Phenolic Content (TPC) ranging from 1000 to 3000 mg of GAE. The experiments were conducted at four locations of FRIM's Sub-station; SPF Mata Ayer (Perlis), SPF Maran (Pahang), SPF Setiu (Terengganu) and FRIM Kepong (Selangor). The description of experimental sites and environmental conditions at four locations trial was shown in Table 1 and 2. A randomized complete block design (RCBD) with four replications for each clones and within three blocks. The intra and inter-row spacing was 0.7 cm and 0.4 cm resulting in 32 plants per block. The planting bed size was 2.0 m x 6.0 m with 0.0014 $m^2$ per plot area. Plants were covered with 90% shade of netting. The data on morphological characteristics of the plants such as plant height, leaf number, leaf length, leaf width and collar region are collected monthly until 9 months after planting.

**TABLE 1**
**DESCRIPTION OF EXPERIMENTAL SITES**

| Meteorological characters | Locations | Years | |
|---|---|---|---|
| | | 2013 (Jan – Dec) | 2014 (Jan – Aug) |
| Rainfall (mm) | Taman Negeri Perlis | 148.6 | 98.5 |
| | PP Kg. Awah | 192.7 | 150.7 |
| | Felda Belera | 119.1 | 126.4 |
| | FRIM Kepong | 56.7 | 39.5 |
| Number of rain days | Taman Negeri Perlis | 9.7 | 10.3 |
| | PP Kg. Awah | 8.4 | 10.6 |
| | Felda Belera | 4.2 | 13.1 |
| | FRIM Kepong | 11.4 | 8.1 |
| 24-Hours temperature (°C) | Taman Negeri Perlis | 23.7 | 26.1 |
| | PPP Kg. Awah | 24.4 | 27.0 |
| | Felda Belera | 17.8 | 26.6 |
| | FRIM Kepong | 26.5 | 26.7 |

***Adopted from: Malaysia Meteorological Department***

**TABLE 2**
**ENVIRONMENTAL CONDITIONS AT FOUR LOCATIONS TRIAL**

| Locations | Parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | Light Intensity (lux) | Relative Humidity (%) | | | Temperature (°C) | | |
| | | Min | Max | Mean | Min | Max | Mean |
| Mata Ayer | 2218.10 | 44.60 | 60.50 | 52.55 | 26.10 | 31.50 | 28.80 |
| Maran | 1130.46 | 79.38 | 93.88 | 86.63 | 24.96 | 28.00 | 26.48 |
| Setiu | 6825.50 | 45.10 | 60.50 | 52.80 | 41.30 | 34.00 | 37.65 |
| Kepong | 5734.21 | 43.25 | 56.28 | 49.76 | 26.59 | 30.38 | 28.49 |

Analysis of variance was carried out for all the traits of eight evaluated *Labisia pumila* var. *alata* genotypes at each individual locations trial and also for combine ANOVA for a complete set of experiments at four locations trial. The purpose of conducted this analysis is to assess variation among environments in each individual locations for experimental error. Besides, this analysis help in estimate and compare the mean value for level of fixed factors such as clones mean value across the locations. The significance of different effects in this study such as locations, clones, block, block within location and interaction between clones of *L. pumila* and environments in locations trial are identified.

## III. RESULTS AND DISCUSSION

### 3.1 Mean growth characteristics and Analysis of Variance (ANOVA)

The results of mean, standard error mean and coefficient variation for all studied traits of eight *L. pumila* var. *alata* genotype at each locations trial; Mata Ayer, perlis; Maran, Pahang; Setiu, Terengganu and Kepong, Selangor were shown in Table 3. Mean for plant height, leaf number, leaf length and leaf width of all evaluated clones showed differences among four locations trial by Duncan's Multiple Range Test at significance level, $P < 0.05$. The plant height at Kepong, Selangor showed higher mean value 5.89cm compared to other three locations that are insignificant for this character. For leaf number and leaf length character, all clones showed significantly differ among four locations trial but both traits gave higher mean value at Maran, Pahang with 4.00 cm and 11.78 cm in respectively. The differences may be due to some external factors like soil series, distribution rainfall, or temperature and internal factor due to clones differences at each locations. Results showed that there is no significant mean value for collar region character in each locations trial. Based on results, all studied characteristics of eight *L. pumila* var. *alata* clones have larger coefficient of variation (CV) which more than 30%. This showed that all studied trait has much more variability relatives to its mean.

**TABLE 3**
**MEAN, STANDARD ERROR MEAN AND COEFFICIENT VARIATION (CV) FOR EVALUATED PLANT GROWTH TRAITS OF EIGHT *LABISIA PUMILA* VAR. *ALATA* CLONES AT FOUR LOCATIONS TRIAL.**

| | Plant Growth Traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | Plant height (cm) | CV (%) | Leaf number (n) | CV (%) | Leaf length (cm) | CV (%) | Leaf width (cm) | CV (%) | Collar region (mm) | CV (%) |
| Mata Ayer | $3.99 \pm 0.18^{b}$ | 44.64 | $3.57 \pm 0.16^{b}$ | 42.53 | $9.88 \pm 0.33^{b}$ | 32.46 | $3.74 \pm 0.12^{b}$ | 31.59 | $2.98 \pm 0.08^{a}$ | 27.12 |
| Maran | $4.39 \pm 0.17^{b}$ | 37.24 | $4.00 \pm 0.14^{a}$ | 34.38 | $11.78 \pm 0.37^{a}$ | 30.34 | $4.27 \pm 0.14^{a}$ | 32.22 | $3.14 \pm 0.07^{a}$ | 21.13 |
| Setiu | $4.03 \pm 0.19^{b}$ | 42.81 | $3.22 \pm 0.15^{c}$ | 43.79 | $9.54 \pm 0.36^{b}$ | 35.35 | $3.67 \pm 0.13^{b}$ | 33.30 | $3.03 \pm 0.07^{a}$ | 22.89 |
| Kepong | $5.89 \pm 0.22^{a}$ | 35.28 | $3.07 \pm 0.15^{c}$ | 47.09 | $11.19 \pm 0.39^{a}$ | 32.56 | $4.10 \pm 0.13^{a}$ | 30.37 | $3.04 \pm 0.09^{a}$ | 30.46 |

***Means followed by the same letter are not significantly different at significance level, P < 0.005***

Results of the combine ANOVA as shown in Table 4 involving four locations with different environments of climate for traits of plant height, leaf number, leaf length, leaf width and collar region.

**TABLE 4**
**COMBINED ANALYSIS OF VARIANCE FOR THE STUDIED TRAITS OF *LABISIA PUMILA* VAR. *ALATA* AT FOUR LOCATIONS TRIAL**

| Source of variation | d.f. | Mean Square | | | | |
|---|---|---|---|---|---|---|
| | | Plant Height (cm) | Leaf number | Leaf length (cm) | Leaf width (cm) | Collar region (mm) |
| Location | 3 | 78.04* | 14.88* | 106.72* | 7.75* | $0.48^{ns}$ |
| Block (Location) | 8 | $3.39^{ns}$ | 4.89* | 23.63* | $1.31^{ns}$ | $0.48^{ns}$ |
| Clone | 7 | 30.63* | 25.19* | 132.56* | 12.80* | 6.48* |
| Location x Clone | 21 | 4.19* | 3.14* | 15.29* | 2.11* | 0.99* |
| Block x Clone (Location) | 56 | 3.70* | $1.49^{ns}$ | 12.28* | 1.86* | 0.68* |
| Error | 266 | 2.40 | 1.44 | 8.22 | 1.21 | 0.41 |

***ns(not significant) , * Significance at P < 0.05***

Location effect was significant for all the characteristics measured except for collar region. There is fluctuation in the growth performance of *L. pumila* var. *alata* for all locations. The differences may be due to the variability in soil type, temperature, relative humidity, light intensity and climate. Effect of block within location showed only leaf number and leaf length has a significant different. It was indicated that the variation in leaf length depend on how it expose to light availability at each block. Fei Xu [5] in the study, stated that the effect of light availability in low light available increase the leaf elongation and lamina areas. In this study, significantly clone main effects indicated there are inconsistent in performance of *L. pumila* var. *alata* between these three locations due to large variance exist between individual clones.

The first-order interaction (location x clone) appeared to be highly significant for most of the measured traits (Table 3). As mentioned by Abd El-Moneim and Cocks (1993), all evaluated clones tended to rank differently when grown at different locations. Second-order interaction, block x clone (location) also gave similar effects on all measured characteristics except leaf number. It shows that all evaluated clones in each location with respected plant growth characteristics response differently at different block.

Inconsistency in the growth performance of *L. pumila* var. *alata* clones across location that existing in this study make difficult for any clones to be released commercially. Identification of the best clones and stable clones adapted to a range of environments also are become complicated.

### 3.2 Stability of *Labisia pumila* var. *alata* Clones For Identification of Elite Planting Materials

In this study, the existence of genotype x environment (GE) interaction raised the need to identify stable and better plant growth. Method proposed by Francis and Kannenburg (1978) was used by plot the mean of plant growth trait versus coefficient of variation (CV) across environments. This plot yields scattergram with four sections resulted from mean of plant growth trait and grand mean. The four sections identified as Group I until Group IV. Group I is a group of clones with high mean trait above grand mean and small CV per cent. Clones in this group were considered has good trait and stable. Whereas, Group II is a group of clones with high mean trait above good trait and large CV showed good trait but has low stability. Group III showed clones with low mean trait and small CV were classified as poor trait but has high stability. Clones with poor trait and unstable were fell within Group IV when the mean trait is below grand mean and large CV per cent present.

The distribution of each clones respected to every evaluated plant growth characters across four locations trial were shown in Fig 1, 2, 3, 4 and 5. The most desirable plants with high mean of plant growth and small variability were selected as stable and identified as potential elite clone

In Fig 1, clones KF07 and KF08 were present in Group I. Both clones was considered stable and has maximum plant height above the grand mean (4.54cm). Clones KF03 and KF02 showed they were on border-line which is have good traits but low in stability. Clone KF05 and KF06 in Group III was judged as unstable due to plant height mean value below the grand mean. Clone KF01 and KF04 were fell within Group IV. Clones in this Group were considered unstable and less performance of plant height due to its large variance.

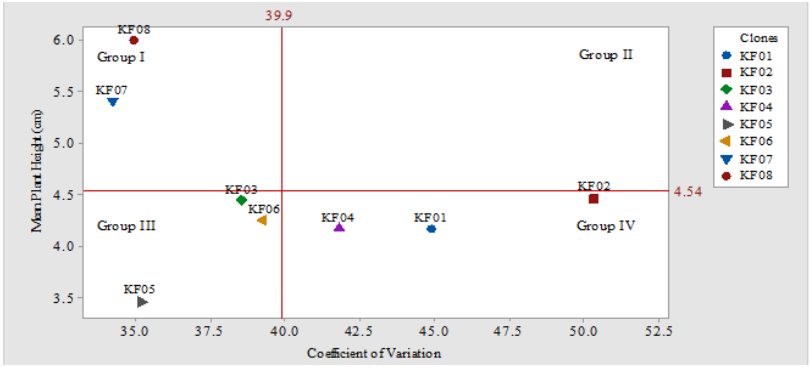


**FIG 1: MEAN PLANT HEIGHT VERSUS COEFFICIENT OF VARIATION FOR EIGHT *LABISIA PUMILA* VAR. *ALATA* CLONES OVER FOUR LOCATIONS TRIAL**

Fig 2 showed that most *L. pumila* var. *alata* clones are most stable with less fluctuation in growth over different locations trial as they fell within Group I but not for clone KF04 that fell within Group II. Clone KF04 has maximum mean leaf number with large variability over locations trial. It means this clone is sensitive to environmental changes and adapted only at specific location.

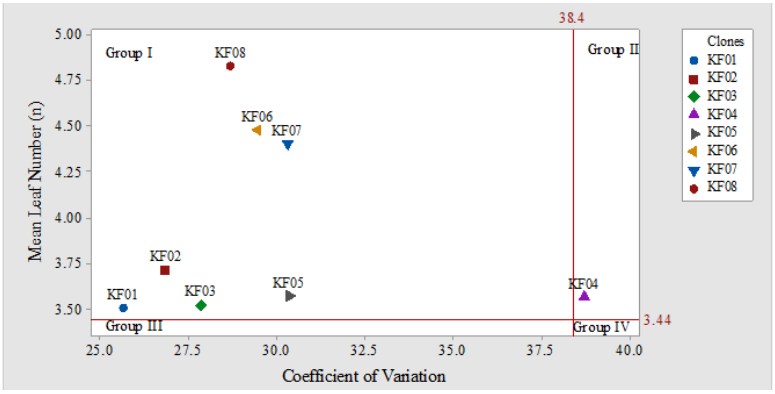


**FIG 1: MEAN LEAF NUMBER VERSUS COEFFICIENT OF VARIATION FOR EIGHT *LABISIA PUMILA* VAR. *ALATA* CLONES OVER FOUR LOCATIONS TRIAL**

The distribution of each clones across four locations trial respected to the leaf length performance has shown in Fig 3. Here, clones KF02, KF07 and KF08 within Group I looks more outperformed than others. They were considered as stable across different environments and had higher mean growth in terms of leaf length. Clone KF03 fell within Group II has maximum mean of leaf length and the variability across the locations trial also are large. This clone would be better adapted to specific environments or locations tested. In Group III, clone KF06 had CV per cent values small than the CV per cent grand mean but still has minimum mean for leaf length. Thus, they were considered unstable and low in growth. Whereas clone KF01 and KF05 were fell within Group IV. They had high CV per cent with minimum leaf length mean that lead to judge them as unstable.

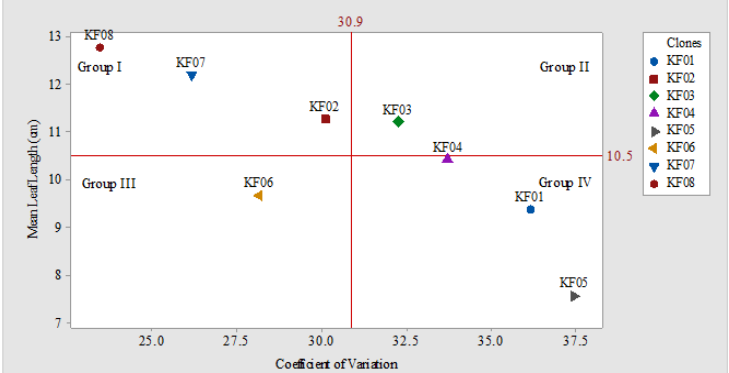


**FIG 3: MEAN LEAF LENGTH VERSUS COEFFICIENT OF VARIATION FOR EIGHT *LABISIA PUMILA* VAR. *ALATA* CLONES OVER FOUR LOCATIONS TRIAL**

For leaf width mean versus coefficient of variation, clone KF08 and KF06 were fell within Group I as the mean leaf width for both clones were in maximum which is 4.82cm and 4.47cm respectively. Clone KF07 was looked more sensitive to environmental changes as it gave CV per cent value more than CV per cent grand mean. Clone KF07 is classified as less stable over locations or environments. Clones KF01, KF02 and KF03 had minimum mean of leaf width and low of CV per cent. So, they fell within Group III and judged as stable. Again, clone KF05 is fell within Group IV same as clone KF04 as they had large CV per cent and minimum mean growth. So, both of these clones are not selected as potential elite clone.

Fig 5 shows only clone KF08 fell within Group I whereas KF06 and KF07 fell within Group II. Even they have maximum mean of collar region, which greater than grand mean of plant growth for this character, Clone KF08 is more stable as the variance is small. Both clones KF06 and KF07 would be selected to be targeted them to specific environments where it may perform well. Clones KF01 and KF02 within Group III had low mean of collar region than the grand mean and CV per cent values below the CV per cent grand mean. Clones fell within this Group were considered having high stability but low in performance, which is minimum collar region are produced.

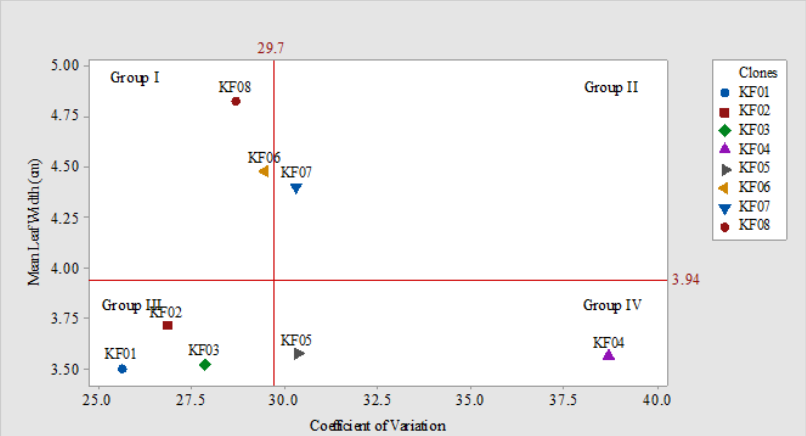


**FIG 4: MEAN LEAF WIDTH VERSUS COEFFICIENT OF VARIATION FOR EIGHT *LABISIA PUMILA* VAR. *ALATA* CLONES OVER FOUR LOCATIONS TRIAL**

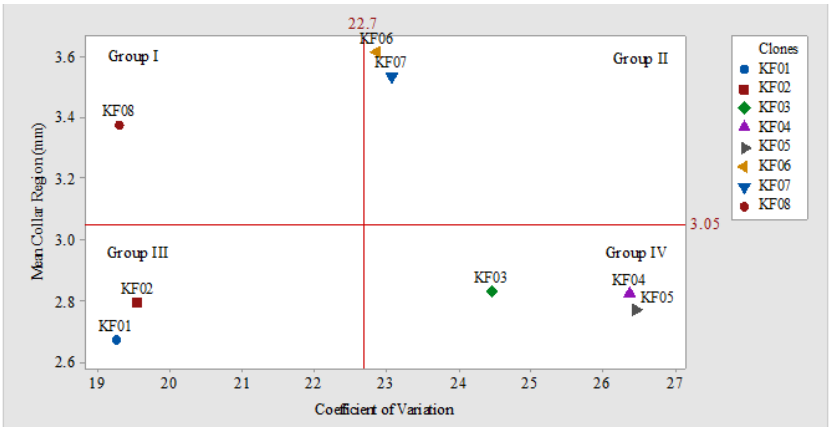


**FIG 5: MEAN COLLAR REGION VERSUS COEFFICIENT OF VARIATION FOR EIGHT *LABISIA PUMILA* VAR. *ALATA* CLONES OVER FOUR LOCATIONS TRIAL**

Based on results obtained from Francis and Kannenburg (1978) method, it was proved that clones KF07 and KF08 were the best among all clones in all of the plant growth characteristics evaluated especially for leaf characters. As results, clones KF07 and KF08 were grow best at all locations trial and exhibit maximum mean with low coefficient of variation

## IV. CONCLUSION

This study showed that KF07 and KF08 were selected as the most desirable and stable clones as they have consistently high mean of performance respected to all the traits studied. Therefore, both clones are identified as elite since they performed well in various regions of environment and geographical locations.

In this study, it was suggested to lengthen the period of planting in field as this species of *L. pumila* has slow growth and achieved their maturity phase at two years old. In addition, the importance of environment selction on growth characteristics in breeding works should be emphasised so that the direct impact to the plant growth (phenotypic) are minimized. The understanding of environmental and genotypic causes of GXE interaction on *L. pumila* is important at all stages of plant breeding. As beneficial, the information about the causes of GXE interaction can be used establish breeding objectives, to identify ideal environmental conditions and to formulate recommendations for areas of optimal plant adaptation

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